S/N UNKNOWN **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Thomas R. Adams et al.	Examiner:	Unknown
Serial No.:	Unknown	Group Art Unit:	Unknown
Filed:	Herewith	Docket:	950.030US2
Title:	TRANSGENIC MAIZE WITH INCREASED MANNITOL CONTENT		

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to taking up the above-identified patent application for review, please amend the application as follows:

IN THE SPECIFICATION

On page 1, line 6, after "This application" please insert -- is a divisional application of U.S. application Serial No. 08/599,714, filed January 19, 1996, allowed, which --.

On page 1, line 7, after "which", please delete "is" and insert --are-- therefor.

IN THE CLAIMS

Please cancel claims 1-58 without prejudice.

Please add the following new claims:

59. (New) A transformed monocot plant, which plant is substantially tolerant or resistant to a reduction in water availability, the cells of which comprise a recombinant DNA segment comprising a preselected DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, wherein the enzyme is expressed in an amount effective to confer tolerance or resistance to the transformed plant to a reduction in water availability.

60. (New) The transformed plant of claim 59 wherein the transformed plant has an improved osmotic potential when the total water potential of the transformed plant approaches zero.

61. (New) A fertile transgenic *Zea mays* plant comprising a recombinant DNA segment comprising a promoter operably linked to a first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, wherein the first DNA segment is expressed so that the level of the enzyme is increased in the transgenic *Zea mays* plant, and wherein the recombinant DNA segment is heritable.

62. (New) The fertile transgenic *Zea mays* plant of claim 61 wherein the recombinant DNA segment further comprises a second DNA segment encoding an amino terminal chloroplast transit peptide operably linked to the first DNA segment.

63. (New) A seed produced by the transgenic plant of claim 61.

64. (New) A method to increase water stress resistance or tolerance in monocot plant cells, comprising:

(a) introducing into cells of a monocot plant an expression cassette comprising a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in the monocot plant cells, to yield transformed monocot plant cells; and

(b) expressing the enzyme encoded by the preselected first DNA segment in the transformed monocot plant cells so as to render the transformed monocot plant cells substantially water stress tolerant or resistant.

65. (New) A method to increase water stress resistance or tolerance in a monocot plant, comprising:

(a) introducing into cells of a monocot plant an expression cassette comprising a preselected DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in the monocot plant cells, to yield transformed monocot plant cells; and

(b) regenerating a differentiated fertile plant from said transformed cells, wherein the enzyme encoded by the preselected DNA segment is expressed in cells of the plant so as to render the transformed monocot plant substantially water stress tolerant or resistant.

66. (New) The method according to claim 65 wherein the expression cassette is introduced into the plant cells by a method selected from the group consisting of electroporation, protoplast transformation, and microprojectile bombardment.

67. (New) The method according to claim 65 wherein the cells of the monocot plant comprise cells of callus, immature embryos, gametic tissue, meristematic tissue or cultured cells in suspension.

68. (New) The method according to claim 65 wherein the expression cassette further comprises a second DNA segment encoding an amino terminal chloroplast transit peptide which is operably linked to the preselected first DNA segment.

69. (New) The method according to claim 68 wherein the second DNA segment encodes a maize chloroplast transit peptide.

70. (New) The method according to claim 68 wherein the enzyme is expressed in the cytosol of the cells of the transformed monocot plant.

71. (New) The method according to claim 68 wherein the enzyme is expressed in the chloroplasts of the cells of the transformed monocot plant.

72. (New) A transformed monocot plant regenerated from the transformed plant cells obtained by the method of claim 68.

73. (New) A transgenic seed of the transformed plant of claim 72.

74. (New) The method according to claim 65 further comprising (c) obtaining progeny from said fertile plant of step (b), which comprise said preselected DNA segment.

75. (New) The method according to claim 74 wherein said progeny are obtained by crossing said fertile plant of step (b) with an inbred line.

76. (New) The method according to claim 74 comprising obtaining seed from said progeny and obtaining further progeny plants comprising said preselected DNA segment from said seed.

77. (New) The method according to claim 76 wherein seeds are obtained from said further progeny plants and plants comprising said preselected DNA segment are recovered from said seed.

78. (New) The method according to claim 75 comprising obtaining seed from said progeny and obtaining further progeny plants comprising said preselected DNA segment from said seed.

79. (New) The method according to claim 78 wherein seeds are obtained from said further progeny plants and plants comprising said preselected DNA segment are recovered from said seed.

80. (New) The method according to claim 75 wherein the progeny obtained in step (c) are crossed back to the inbred line, to obtain further progeny which comprise said preselected DNA segment.

81. (New) The method according to claim 80 wherein said further progeny are crossed back to the inbred line to obtain progeny which comprise said preselected DNA segment.

82. (New) An expression cassette comprising a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in a host cell, wherein the promoter is selected from the group consisting of the *Glb* promoter, the *AdhI* promoter, and the *ActI* promoter.

83. (New) The expression cassette of claim 82 further comprising a second DNA segment encoding an amino terminal chloroplast transit peptide which is operably linked to the preselected first DNA segment.

84. (New) The expression cassette of claim 83 wherein the chloroplast transit peptide is a maize chloroplast transit peptide.

85. (New) The expression cassette of claim 82 which further comprises an enhancer element.

86. (New) The expression cassette of claim 85 wherein the enhancer element is subject to tissue-specific regulation.

87. (New) The expression cassette of claim 82 which further comprises a selectable marker gene or a reporter gene.

88. (New) An expression cassette comprising
(a) a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline operably linked to a promoter functional in a host cell; and
(b) a second DNA segment that encodes an untranslated regulatory element, wherein the second DNA segment separates the preselected DNA segment from the promoter.

89. (New) The expression cassette of claim 88 wherein the untranslated regulatory element is the *Adh*I intron 1.

90. (New) The expression cassette of claim 88 wherein the promoter is turgor-inducible.

91. (New) The expression cassette of claim 88 wherein the promoter is abscisic acid inducible.

92. (New) The expression cassette of claim 88 wherein the promoter is developmentally regulated.

93. (New) The expression cassette of claim 88 wherein the promoter is a constitutively expressed promoter.

94. (New) The expression cassette of claim 88 wherein the promoter is subject to tissue-specific regulation.

95. (New) The expression cassette of claim 88 wherein the promoter is water-stress inducible.

96. (New) An expression cassette comprising:

(a) a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline operably linked to a promoter functional in a host cell; and

(b) a second DNA segment encoding a maize chloroplast transit peptide, wherein the second DNA segment is operably linked to the preselected first DNA segment.

REMARKS

Claims 1-58 have been canceled without prejudice. New claims 59-96 have been added. Claims 59-96 are pending.

New claim 59 is supported by originally-filed claim 32.

New claim 60 is supported by originally-filed claim 33.

New claim 61 is supported by originally-filed claim 43.

New claim 62 is supported by originally-filed claim 44.

New claim 63 is supported by originally-filed claim 46.

New claim 64 is supported by originally-filed claim 22.

New claim 65 is supported by originally-filed claim 31.

New claims 66-73 are supported by originally-filed claims 23-30, respectively.

New claims 74-81 are supported by originally-filed claims 51-58, respectively.

New claim 82 is supported by originally-filed claim 1.

New claims 83-87 are supported by originally-filed claims 8-12, respectively.

New claim 88 is supported by originally-filed claim 13.

New claims 89-95 are supported by originally-filed claims 14-20, respectively.

New claim 96 is supported by originally-filed claim 21.

The osmoprotectant proline is supported in the specification at page 1, lines 26-28; at page 2, lines 15-17; and at page 30, lines 28-29.

CONCLUSION

Applicant believes the claims are in condition for allowance and respectfully requests allowance of the claims. The Examiner is invited to telephone the below-signed attorney at (612) 373-6903 to discuss any questions which may remain with respect to the present application.

Respectfully submitted,

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